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Original Article

Effects of anti-Müllerian hormone and follicle stimulating hormone levels on *in vitro* fertilization pregnancy rate

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ABSTRACT

Objectives: To analyze the relationship between *in vitro* fertilization (IVF) pregnancy rate and basal serum hormone levels before patients begin an IVF course.
Materials and methods: In this retrospective study, we analyzed patients with anti-Müllerian hormone (AMH) data and IVF data from January 2009 to October 2012. Pregnancy rates were calculated by AMH and follicle stimulating hormone quartiles and analyzed using the independent samples *t* test. Furthermore, patients were divided into three groups by age. The Chi-square test was used to assess the association between the parameters and IVF pregnancy rates.
Results: From the 910 IVF treatment courses, 377 (41.4%) clinical pregnancies resulted. The pregnant and nonpregnant groups differed significantly in age and FSH and AMH levels. The pregnancy rate was 53.3% for patients aged <32 years and 22.1% for patients aged >38 years. The pregnancy rate was 53.4% for patients with FSH levels <5.6 mIU/mL and 25.8% for patients with FSH levels >8.9 mIU/mL. The pregnancy rate was 56.8% for patients with AMH levels >4.0 ng/mL and 20.0% for patients with AMH levels <1.1 ng/mL. Furthermore, among patients aged <40 years, AMH and FSH were significantly associated with pregnancy rate. Higher pregnancy rates were found among the groups with higher AMH levels than in groups with lower AMH levels.
Conclusion: For patients aged <40 years, basal serum AMH level and FSH level affected the IVF pregnancy rate, and patients with higher AMH levels had better pregnancy rates.

Introduction

Treatment for sterility entered a new era after the first baby conceived by *in vitro* fertilization (IVF) was born in 1978 [1]. The rush to control the female reproductive cycle created the development of reproductive medicine and assisted reproductive technology, based on academic reproductive endocrinology [2].

The female menstrual cycle is regulated by a series of hormones, including gonadotropin-releasing hormone, follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), and progesterone. Estrogen and progesterone regulate follicle growth from the start of follicle development through regulation of the *corpus luteum* [3]. FSH plays a role in primordial follicle survival [4]. When the dominant follicles appear, there is a transformation of dependency from FSH to LH. Through altering LH receptor mRNA expression, LH-responsiveness of theca and granulosa cells during follicular development changes. Furthermore, the LH surge triggers ovulation. The interactions between extraovarian and intraovarian factors determine the fate of the follicle and the quality of the oocyte [5].

Intervention in the menstrual cycle by induction of the events leading to egg production and possible fertilization upon introduction of sperm, IVF, is costly to women in terms of physiological and psychological distress, not to mention the financial costs of resources required to complete IVF [6]. Except for multiple gestations in one pregnancy, the side effects of IVF include complications of surgery and ascites due to ovarian hyperstimulation syndrome

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Table 1
Patient characteristics in pregnant and nonpregnant patients.

	Nonpregnant ($n = 533$)	Pregnant ($n = 377$)	p*
Age (y)	36.2 ± 4.7	34.0 ± 3.9	<0.001
FSH (mIU/mL)	8.7 ± 5.3	6.9 ± 2.6	<0.001
LH (mIU/mL)	5.2 ± 3.7	5.1 ± 3.7	0.435
E2 (pg/mL)	37.7 ± 22.5	38.2 ± 31.3	0.228
AMH (ng/mL)	2.6 ± 3.1	3.9 ± 3.6	<0.001

*$p < 0.05$ is considered statistically significant.
AMH = anti-Müllerian hormone; E2 = serum estradiol during the follicular phase of menses; FSH = follicle stimulating hormone; LH = luteinizing hormone.

and even death [7]. Therefore, it is necessary to determine the prognostic factors predictive of IVF pregnancy success before patients begin an IVF course.

Anti-Müllerian hormone (AMH), produced by granulosa cells of preantral follicles, restricts folliculogenesis by an inhibitory effect on FSH sensitivity [8]. As a result, AMH is considered a marker for assessing ovarian reserve [9]. Clinically, AMH is used as the predictive factor for ovarian response and IVF pregnancy rate. Many published papers discuss AMH as the predictive factor for IVF pregnancy success and ovarian hyperstimulation syndrome. Nonetheless, the predictive results for AMH are inconclusive [10,11].

The purpose of this retrospective study was to assess the relationship between IVF pregnancy rate and AMH level. We also analyzed the relationships between IVF pregnancy rate and basal serum hormone levels (FSH, LH, and E2) before patients began an IVF course.

Materials and methods

The IVF treatment course records of 1003 patients with AMH data were retrieved from the Department of Obstetrics and Gynecology, Chang Gung University College of Medicine, Linkou Medical Center, Taoyuan, Taiwan from January 2009 to October 2012. Ninety-three (9.27%) treatment courses without embryo transfer, unrelated to ovarian reserve and embryo quality, were excluded from this study. Four (0.40%) treatment courses were from oocyte donors. No sperm was found after microsurgical epididymis sperm aspiration or testicular sperm aspiration in six (0.60%) treatment courses. There were 20 (1.99%) treatment courses for preimplantation genetic diagnosis, with genetically abnormal embryos for embryo transfer. Three (0.30%) patients dropped out due to individual problems. All of these IVF courses were excluded. Another 56 (5.58%) patients requested oocyte or embryo cryopreservation. Severe ovarian hyperstimulation occurred in three patients (0.30%). One patient (0.10%) had internal bleeding after oocyte retrieval. These IVF courses were also excluded.

To consider the effect of ovarian function, we included other treatment courses in which there was little ovarian response, no oocyte retrieved, no fertilized embryo, poor embryo quality, or arrested embryo development during culture. As a result, 910 IVF treatment courses were included, and all embryo transfers, when done, were fresh.

Serum samples were collected during the follicular phase of the menstrual cycle. FSH, LH, E2, and AMH were measured in all patients.

AMH levels were measured using a two-step immunological sandwich type assay for the *in vitro* determination of AMH/Müllerian inhibiting substance in human serum and plasma (IMMUNOTECH, Beckman Coulter, Marseille, France). The lowest AMH concentration significantly different from the zero calibrator was 0.14 ng/mL. The intra-assay and interassay coefficients of variation were ≤12.3% and ≤14.2%, respectively. The measurement range of AMH was 0.14–21 ng/mL.

The definition of pregnancy in this study was clinical pregnancy, with ultrasound proof of one or more intrauterine gestational sacs. This study was approved by the Research Ethics Committee of Chang Gung Memorial Hospital, Taoyuan County, Taiwan (Institutional Review Board No. 101-4814B). The need for informed consent was waived due to the retrospective nature of the study.

Statistical analysis

Data are presented as mean ± standard deviation and percentage. The independent samples t test was used to compare the statistical relationships of all parameters between the pregnancy and nonpregnancy groups. The pregnancy rate was calculated by quartiles of the statistically significant AMH and FSH levels. Furthermore, patients were divided into three groups by age, and the Chi-square test was used to assess the relationship between pregnancy rate and AMH and FSH levels. SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. A p value <0.05 was considered statistically significant.

Results

From the 910 IVF courses, 377 (41.4%) clinical pregnancies resulted. The mean age in the pregnant group was 34.0 ± 3.9 years and 36.2 ± 4.7 years in the non-pregnant group. FSH was 6.9 ± 2.6 mIU/mL and 8.7 ± 5.3 mIU/mL whereas AMH was 3.9 ± 3.6 ng/mL and 2.6 ± 3.1 ng/mL in the pregnant and nonpregnant groups, respectively. Age and FSH and AMH levels differed significantly between the pregnant and nonpregnant groups (Table 1). Compared with the nonpregnant group, patients in the pregnant group were younger, and had a lower FSH level, and a higher AMH level.

When pregnancy data were analyzed by age quartiles, the pregnancy rate ranged from 53.3% in the age <32 years group to 22.1% in the >38 years group ($p < 0.001$). Similarly, in the group with AMH >4.0 ng/mL, the pregnancy rate was 56.8% whereas it was 20.0% in the group with AMH <1.1 ng/mL ($p < 0.001$; Table 2). Comparably, the pregnancy rate ranged from 53.4% in the group with FSH <5.6 mIU/mL to 25.8% in the group with FSH >8.9 mIU/mL ($p < 0.001$; Table 2).

Patients were divided into three groups by age: <35 years ($n = 406$); 35–40 years ($n = 345$); and >40 years ($n = 159$). Patients aged <35 years with AMH >4.0 ng/mL had a pregnancy rate of 60.4% whereas the pregnancy rate was 28.1% in the group with

Table 2
Pregnancy rates by age, follicle stimulating hormone (FSH), and anti-Müllerian hormone (AMH) quartiles.

Quartile	<25%		25–50%		50–75%		>75%	
	Range	Pregnancy rate (%)	Range	Pregnancy rate (%)	Range	Pregnancy rate (%)	Range	Pregnancy rate (%)
Age (y)*	<32 ($n = 182$)	53.3	32–35 ($n = 224$)	49.6%	35–38 ($n = 223$)	48.0	>38 ($n = 281$)	22.1
FSH (mIU/mL)*	<5.6 ($n = 221$)	53.4	5.6–7.1 ($n = 220$)	45.5%	7.1–8.9 ($n = 236$)	41.9	>8.9 ($n = 233$)	25.8
AMH (ng/mL)*	<1.1 ($n = 230$)	20.0	1.1–2.2 ($n = 230$)	40.9%	2.2–4.0 ($n = 221$)	48.4	>4.0 ($n = 229$)	56.8

*$p < 0.001$.

Table 3
Pregnancy rates by anti-Müllerian hormone (AMH) level among different age groups.

Age (y)	AMH range (ng/mL)	Pregnancy rate (%)	AMH range (ng/mL)	Pregnancy rate (%)	AMH range (ng/mL)	Pregnancy rate (%)	AMH range (ng/mL)	Pregnancy rate (%)
<35*	<1.1 ($n = 57$)	28.1	1.1–2.2 ($n = 106$)	48.1	2.2–4.0 ($n = 109$)	55.0	>4.0 ($n = 134$)	60.4
35–40*	<1.1 ($n = 102$)	19.6	1.1–2.2 ($n = 80$)	41.3	2.2–4.0 ($n = 83$)	48.2	>4.0 ($n = 80$)	58.8
>40**	<1.1 ($n = 71$)	14.1	1.1–2.2 ($n = 44$)	22.7	2.2–4.0 ($n = 29$)	24.1	>4.0 ($n = 15$)	13.3

*$p < 0.001$.
**$p < 0.506$.

Table 4
Pregnancy rates by follicle stimulating hormone (FSH) among different age groups.

Age (y)	FSH range (mIU/mL)	Pregnancy rate (%)	FSH range (mIU/mL)	Pregnancy rate (%)	FSH range (mIU/mL)	Pregnancy rate (%)	FSH range (mIU/mL)	Pregnancy rate (%)
<35*	<5.6 ($n = 119$)	57.1	5.6–7.1 ($n = 113$)	49.6	7.1–8.9 ($n = 94$)	57.4	>8.9 ($n = 80$)	37.5
35–40**	<5.6 ($n = 77$)	59.7	5.6–7.1 ($n = 78$)	46.2	7.1–8.9 ($n = 98$)	35.7	>8.9 ($n = 92$)	25.0
>40***	<5.6 ($n = 25$)	16.0	5.6–7.1 ($n = 29$)	27.6	7.1–8.9 ($n = 44$)	22.7	>8.9 ($n = 61$)	11.5

*$p < 0.026$.
**$p < 0.001$.
***$p < 0.236$.

AMH <1.1 ng/mL ($p < 0.001$; Table 3). In the age 35–40 years group, pregnancy rates ranged from 58.8% for patients with AMH >4.0 ng/mL to 19.6% in the group with AMH <1.1 ng/mL ($p < 0.001$). The pregnancy rate of patients aged >40 years ranged from 13.3% to 24.1%, depending on the AMH level ($p = 0.506$; Table 3).

Patients aged <35 years with FSH levels of <5.6 mIU/mL had a pregnancy rate of 57.1% whereas the pregnancy rate was 37.5% in the group with FSH >8.9 mIU/mL ($p = 0.026$; Table 4). Patients aged 35–40 years with FSH levels <5.6 mIU/mL had a pregnancy rate of 59.7% whereas the pregnancy rate was 25.0% in the group with FSH levels >8.9 mIU/mL ($p < 0.001$; Table 4). Patients aged >40 years had pregnancy rates ranging from 27.6% to 11.5% depending on the FSH level ($p = 0.236$; Table 4).

Discussion

Prediction of IVF pregnancy rates using serum AMH levels is a controversial issue. Some authors concluded that AMH levels are associated with pregnancy rates [10,12–14] whereas others concluded that they are not [11,15–18]. In our study, based on 377 clinical pregnancies, the mean age in the pregnant group was 34.0 ± 3.9 years, FSH was 6.9 ± 2.6 mIU/mL, LH was 5.1 ± 3.7 mIU/mL, E2 was 38.2 ± 31.3 pg/mL, and AMH was 3.9 ± 3.6 ng/mL. The age and FSH and AMH levels differed significantly between the pregnant and nonpregnant groups (Table 1). The patients aged <32 years had a 53.3% pregnancy rate whereas the group aged >38 years had a 22.1% pregnancy rate. Younger patient groups had better pregnancy rates than older patient groups (Table 2). Among the patient group with AMH >4.0 ng/mL, the pregnancy rate was 56.8%; it was 20.0% in the group with AMH <1.1 ng/mL (Table 2). Higher pregnancy rates were found in the groups with greater AMH levels. In the group with FSH <5.6 mIU/mL, the pregnancy rate was 53.4%; it was 25.8% in the group with FSH >8.9 mIU/mL (Table 2). Higher pregnancy rates were found in the groups with lower FSH levels.

Age influenced the relationship between AMH and IVF pregnancy outcome [19], so patients were divided into three groups by age. Patients, aged <35 years with higher AMH levels had greater pregnancy rates (60.4–28.1%) than older patients with lower AMH levels. A similar pattern was found in the group aged 35–40 years (pregnancy rate range, 58.8–19.6%). Among patients aged >40 years, pregnancy rate did not differ significantly based on AMH level (Table 3). Thus, AMH level could be used as a predictive factor of IVF pregnancy rate for patients aged <40 years. Furthermore, among patients aged <40 years, FSH levels also influenced IVF pregnancy rates. Patients, with lower FSH levels typically had higher pregnancy rates (Table 4), even though among patients aged < 35 years, the group with FSH = 7.1–8.9 mIU/mL had the highest pregnancy rate. This could be due to variations of FSH during the menstrual cycle. Similar to pregnancy rates associated with AMH levels, pregnancy rates associated with FSH levels in patients aged >40 years did not differ significantly.

Based on our results, among patients aged >40 years, the uterus played a more pivotal role than the ovaries did in influencing IVF pregnancy rates. To confirm this hypothesis, additional studies to evaluate endometrial receptivity, such as endometrial morphology by ultrasound [20], biomarkers by lipidomics [21], and genomic signature by endometrial receptivity array [22] have yet to be investigated.

The reduction in ovarian reserve is a physiological process occurring in the late reproductive period, and it is consistently associated with a decrease in AMH levels [23]. As a result, patients with low AMH levels were urged to undergo IVF treatment as soon as possible. Furthermore, for patients with low AMH levels, clinicians had to find effective interventions to improve ovarian response, such as dehydroepiandrosterone [24,25], transdermal testosterone [26], and visfatin [27] treatments.

For patients with poor ovarian response, ovum donation is the only proven method of achieving pregnancy in patients with primary ovarian insufficiency or premature ovarian failure [28]. Nonetheless, several of these patients became pregnant after cyclic estrogen/progesterone [29–31], exogenous gonadotropin [32], and gonadotropin-releasing hormone antagonist therapies [33], and spontaneously [34,35]. More clinical trials are required to determine the best method to treat patients with primary ovarian insufficiency or premature ovarian failure.

In conclusion, for patients aged <40 years, basal serum AMH level and FSH level affected the IVF pregnancy rate, and patients with higher AMH levels had higher pregnancy rates. To improve the clinical benefits of AMH, more interventions for patients with low AMH levels need to be investigated.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

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